·论著(译文)·

Levels of Interleukin-8 and Tumor Necrosis Factor in the Cerebrospinal Fluid in Children with Purulent Meningitis and Viral Encephalitis

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Abstract: Objective To detect the levels of interleukin-8 (IL-8) and tumor necrosis factor (TNF) in the cerebrospinal fluid (CSF) in children with purulent meningitis (PM) and viral encephalitis (VE). **Methods** Levels of IL-8 and TNF in CSF were determined using ELISA in 20 children with (PM), 17 children with viral meningoencephalitis (VME), 25 children with (VE), and 15 normal children who served as the controls. **Results** The levels of CSF IL-8 and TNF in the PM group [(443 ±247) ng/L and (640 ±283) ng/L] were significantly higher than those in the VME group [(184 ±86) ng/L and (154 ±95) ng/L], VE group [(54 ±36) ng/L and (30 ±26) ng/L] and control group [(27 ±20) ng/L and (24 ±21) ng/L] ($P < 0.01$). The levels of CSF IL-8 and TNF in the VME group were also significantly higher than those in the VE group and control group ($P < 0.01$). CSF IL-8 levels in the VE group were higher than those in the control group ($P < 0.05$), but there was no significant elevation in CSF TNF concentrations. A significantly positive correlation was found between IL-8 and TNF in the PM and VME groups ($r = 0.682$, $P < 0.01$ and $r = 0.534$, $P < 0.05$, respectively). CSF IL-8 levels were significantly related to neutrophil counts in patients with PM ($r = 0.777$, $P < 0.01$), but no significant relationship was shown between CSF IL-8 levels and neutrophil counts in children with VME and VE. There was no correlation between CSF TNF concentrations and neutrophil counts in the three patient groups. Nor was there any correlation between CSF IL-8 and TNF levels and other inflammatory parameters including CSF mononuclear cell counts, and protein and sugar levels in the three patient groups. **Conclusions** IL-8 and TNF are important mediators in the meningeal inflammatory process in children with meningitis. The determination of IL-8 and TNF levels may be valuable in the diagnosis of purulent meningitis.

Key words: Meningitis; Encephalitis; Cerebrospinal fluid; Interleukin-8; Tumor necrosis factor

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Recent studies have confirmed that there is a close correlation between the nervous system and the immune system. The infection of the central nervous system (CNS) may be accompanied by the change of cytokines. The goal of this study was to detect the levels of interleukin-8 (IL-8) and tumor necrosis factor (TNF) in the cerebrospinal fluid (CSF) from patients with purulent meningitis (PM), viral meningoencephalitis (VME) and viral encephalitis (VE), and to evaluate the relationship among IL-8, TNF and several CSF inflammatory parameters.

1 Materials and Methods

1.1 Patients

A prospective study was conducted on 77 patients with clinically suspected meningitis or encephalitis. Every patient received a diagnostic lumbar puncture on admission.

The PM group included 20 patients (13 males, 7 females; aged between 18 days and 13 years), with the duration of illness before admission ranging from 1 to 5 days. The diagnosis of PM was established by

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the typical clinical symptoms, routine and biochemical examinations, bacterial culture in CSF, and the rapid response to antibiotic therapy. The etiologic agents in the 14 patients with positive CSF cultures were: Staphylococcus (n = 6), Streptococcus pneumoniae (n = 4), Escherichia coli (n = 3), and Moraxella atlantae (n = 1); the infecting organisms in the remainders with negative CSF and positive blood cultures included: Staphylococcus aureus (n = 3), Staphylococcus epidermidis (n = 1), and Streptococcus pneumoniae (n = 2). The VE group comprised 25 patients (14 males, 11 females; aged between 5 months and 14 years), with the duration of illness before admission ranging from 1 to 8 days. The diagnosis of VE was based on the typical clinical symptoms, routine and biochemical examinations in CSF, detection of IgM antibodies, electroencephalogram, and craniocerebral CT or MRI.

The VME group contained 17 patients (11 males, 6 females aged between 9 months and 14 years). The diagnotor of VME was similar to that of VE. The control group consisted of 15 patients (10 males, 5 females; aged between 3 months and 13 years), who had the indication of lumbar puncture, and were then excluded from CNS infection by routine and biochemical examinations in CSF, bacterial culture, the detection of IgM antibodies, electroencephalogram, and craniocerebral CT or MRI. Of the 15 patients, 5 had acute gastroenteritis, 5 upper respiratory infection, 3 hyperpyretic convulsion, and 2 hypocalcemic convulsion.

1.2 CSF collection and determination of IL-8 and TNF

A total of 77 CSF samples were obtained from the patients by lumbar puncture on the first day of hospitalization, and immediately assayed for routine and biochemical examinations, bacterial culture, and the detection of IgM antibodies. The other samples were centrifuged at 2000 g for 10 minutes, and the supernatants were stored at - 20 ℃ until being tested. The levels of IL-8 and TNF in CSF were determined with a double monoclonal antibody ELISA, and the kits were provided by the Department of Immunology of the Fourth Military Medical University.

1.3 Statistical analysis

The levels of IL-8 and TNF in CSF were graphically displayed as Mean ±SD, and the values between the different groups were compared with Student's t test. Correlations among IL-8, TNF and CSF inflammatory parameters (leukocyte count, neutrophil and mononuclear cell counts, and protein and glucose concentrations) were assessed with a multiple regression analysis based on stepwise selection. The data were considered as statistically significant ($P < 0.05$).

2 Results

2.1 CSF IL-8 and TNF levels

The CSF IL-8 and TNF levels for each group are shown in Table 1. The levels of IL-8 and TNF in the PM group were significantly higher than those in the VME, VE and control groups ($P < 0.01$). The IL-8 and TNF levels in the VME group were also markedly higher than those in the VE and control groups ($P < 0.01$). The IL-8 levels in the VE group were higher than those in the control group ($P < 0.05$), but there was no significant elevation in TNF concentrations compared with the controls.

Table 1 CSF IL-8 and TNF levels in different groups ($\bar{x} \pm s$, ng/L)

Group	Number of Cases	IL-8	TNF
Control	15	27 ±20	24 ±21
VE	25	54 ±36[a]	30 ±26
VME	17	184 ±86[b]	154 ±95[b,c]
PM	20	443 ±247[b,c,d]	640 ±283[b,c,d]

a vs control, $P < 0.01$; b vs control group, $P < 0.01$; c vs VE group, $P < 0.01$; d vs VME group, $P < 0.01$

2.2 Correlation between IL-8 and TNF levels

A significantly positive correlation was found between IL-8 and TNF in the PM and VME groups ($r = 0.682$, $P < 0.01$ and $r = 0.534$, $P < 0.05$, respectively), but there was no significantly positive correlation in the VE group ($r = 0.122$, $P > 0.05$).

2.3 Correlation among IL-8, TNF and CSF inflammatory parameters

CSF IL-8 levels were significantly correlated with neutrophil counts in patients with PM (r =

0.777, $P < 0.01$), but no significant relationship was found between IL-8 levels and mononuclear cell counts, and protein or glucose concentrations. There was no correlation between CSF IL-8 levels and neutrophil and mononuclear cell counts, and protein or glucose levels in the VME and VE groups, and nor was there any correlation between CSF TNF levels and inflammatory parameters in the PM, VME and VE groups.

3 Discussion

IL-8 is a chemotactic factor for the migration of neutrophils, T-lymphocytes and basophils. Many cell types, including monocytes, macrophages, T-lymphocytes, neutrophils, vascular endothelial cells and astrocytes, can synthesize and release large amounts of IL-8 when stimulated with IL-1, TNF, and bacterial and viral infections. In CNS, IL-8 is mainly produced by vascular endothelial cells, macrophages, astrocytes and microglial cells[1]. IL-8 is able to induce the infiltration of neutrophils and lymphocytes, and enhance the permeability of blood vessels. The experiments in vitro have confirmed that IL-8 could promote neutrophil degranulation and phagocytosis, stimulate the elevation of intracellular calcium ion, and initiate the release of superoxide ion[2]. Therefore, IL-8 plays an important role in the regulation of inflammation and immunization. Although foreign authors have investigated CSF IL-8 levels in patients with CNS infection, the reported results were variant. Seki et al[3] observed high levels of CSF IL-8 in 7 patients with bacterial meningitis but in none of the 11 patients with viral meningitis. Furthermore, López-Cortés and colleagues[4] analyzed IL-8 concentrations in 70 CSF samples from patients with meningitis of different etiologies, including pyogenic and viral meningitis, self-resolving aseptic meningitis without a special diagnosis, and meningitis of other etiologies, and in 34 normal CSF samples; IL-8 levels were remarkably higher in patients with pyogenic meningitis than those in all other meningitis groups together and individually.

The present study showed that CSF IL-8 levels were obviously elevated in the PM and VME groups, especially in the PM group, and this is similar to what López-Cortés et al found. Since IL-8 concentrations were also increased in the CSF of the patients with VE, it is suggested that IL-8 might be involved in the pathophysiology of meningitis and encephalitis. There was a significantly positive relationship between CSF IL-8 levels and neutrophil counts, but not in mononuclear cell counts from the cases with PM, indicating that IL-8 could be a major chemokine for the immigration of neutrophils into the subarachnoid space from the peripheral circulation in purulent meningitis. CSF IL-8 levels were not related to neutrophil counts in patients with VME, and this may be because VME is characterized by a recruitment of monoclear cells.

TNF is a pleiotropic cytokine produced predominantly by activated macrophages. Although TNF serves to mediate and regulate immune and inflammatory responses, and to participate in anti-infectious response, its overproduction or continuous release would be involved in the pathogenesis of certain diseases, resulting in fever, shock, cachexia, and so on. Our results agree with those of some domestic authors[5,6] in that there was an obvious elevation of CSF TNF levels in the patients with VME and PM in particular, further supporting that TNF is pivotal in the development and progression of meningitis. TNF concentrations in the CSF of the patients with VE did not differ from those in the control subjects, suggesting that TNF may be chiefly involved in regional inflammation of meninges.

In the present study, we found that there existed a significantly positive correlation between IL-8 and TNF concentrations in the CSF of the patients with PM and VME. These data indicate that IL-8 and TNF could mutually induce and enhance, and constitute a network of inflammatory cytokines in the pathophysiology of PM and VME, together with other cytokines, e.g., IL-1, IL-6 and the like.

The data abtained have demonstrated that TNF is in the van of pathogenesis of PM. After bacterial infection, TNF is the first to be detected, and IL-1, IL-6 and IL-8 are the next[2]. Astrocytes and microglia are initially invaded by infectious agents in the early stage of meningitis[7], and release other cytokines, including IL-6 and IL-8, by the induction of

proinflammatory cytokines such as TNF and IL-1. The effects of these cytokines on CNS could not only cause fever and anti-infective response, but also take part in the damage of the brain and the breakdown of the blood brain barrier, resulting in the inflammation of meninges and even the encephalon. Meanwhile, neutrophils in the peripheral circulation are attracted by the chemokine IL-8 in the CSF, and then migrate into the subarachnoid space through the damage of the blood brain barrier[8].

In conclusion, IL-8 and TNF levels were substantially higher in the CSF of patients the with PM than those in the patients with VME and VE, and there was a significant relationship between CSF IL-8 levels and neutrophil counts in the patients with PM. This investigation indicates that the detection of IL-8 and TNF levels may be valuable in the diagnosis of purulent meningitis.

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